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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: Leonid A. Yakubov

Serial No.: 09/753,892 **Group No.:** 1632

Filing Date: January 3, 2001 **Examiner:** Scott David Priebe

For: COMPOSITIONS COMPRISING GENOME SEGMENTS AND METHODS OF USING THE SAME

DECLARATION OF DR. LEONID A. YAKUBOV PURSUANT TO 37 CFR § 1.132

I, Dr. Leonid A. Yakubov, declare as follows:

1. I am the inventor in the above-identified patent application.

2. A copy of my *curriculum vitae* is attached as Exhibit 1.

3. I have reviewed the Official Action dated October 25, 2002 for the above-identified patent application.

4. I have performed and/or supervised the performance of experiments described herein.

5. Experiments were performed with a mouse tumor model to determine if cancer growth can be inhibited *in vivo* by the administration of genomic DNA fragments in accordance with the disclosure in the above-identified application. Mouse A/He strain-specific hepatoma AI and syngenic A/He mice were used in experiment. 1×10^6 cells of the tumor were inoculated intramuscularly into the rear leg of the mice. The tumor became palpable after 7-10 days and could be measured in 2 weeks after the inoculation. Six days after the hepatoma cells inoculation mice started to receive daily injections of either a physiological salt solution (negative control), normal fragmented human genomic DNA (Pana H), or allogenic fragmented murine genomic DNA (Pana A). Human placenta was used as the DNA source for Pana H; liver, kidney and spleen from CBA mice were used as

the DNA source for Pana A. 100 mg of genomic DNA from either human or mouse was isolated using published phenol-free method (Sambrook J, Fritsch EF, Maniatis T: *Molecular cloning: a Laboratory Manual.* Cold Spring Harbor Lab. Cold Spring Harbor, NY; 1989.) and then mildly sonicated to obtain DNA fragments with the length mainly 200 – 3000 bp. Resulting fragment compositions comprising collectively entire human or mouse genomes were administered. The data from the experiment are shown in Exhibit 2. The data demonstrate that both genomic DNA treatments inhibited tumor growth but to a different extent. The administration of the murine DNA inhibited tumor growth 4-fold as compared to the saline control, while the human DNA inhibited tumor growth 2-fold as compared to the saline control.

6. Experiments were performed to determine if a caspase-3 mutation in cultured cells could be repaired *in vitro* using preparations of genomic DNA fragments in the form of fragment compositions comprising collectively entire genomes in accordance with the disclosure in the above-identified application. The cultured cells used were human breast adenocarcinoma MCF-7 cells, which have a 47 bp deletion in caspase-3 gene. Caspase-3 is a key enzyme in the apoptotic cascade. The mutation prevents MCF-7 cells from developing characteristic features of apoptosis, such as plasma membrane shrinkage and formation of apoptotic bodies followed by fragmentation of the MCF-7 genomic DNA. The genomic DNA fragments used were human placenta DNA fragments that were prepared by mild sonication of the DNA in ultrasonic water bath to the size 200-3000 bp. Human placenta DNA fragments were incubated with MCF-7 cells in cell culture medium RPMI 1640 supplemented with 5% FCS at a concentration of 0.3 mg/ml of the DNA for a period of time. At periodical repassaging of the cells approximately once per week fresh medium with supplements and the DNA fragments at the same concentration were added. After 5 and 40 days of incubation with human placenta DNA fragments, the MCF-7 genomic DNA was analysed. Specific amplification of the 48bp deletion region in exon 3 of the caspase-3 gene where the mutation is located was done using primers flanking the region. Two primers were selected: A1 – AAAGATCATACATGGAAGCGAATCAAT (nucleotides +54 - +80 of the exon 3, and A2 – CAGTGCTTTTATGAAAATTCTTATTAT (nucleotides +152 - +178 of the exon 3). The data from the experiment are shown in Exhibit 3. After 5 days the wild-

type band appeared and became more prominent after 40 days. The treated cells also began to display features characteristic of apoptosis, such as chromatin fragmentation in the presence of an apoptosis inducer (TNF-alpha). Untreated cells (cells not treated with fragmented genomic DNA) do not display these features characteristic of apoptosis, such as chromatin fragmentation in the presence of an apoptosis inducer. No fragmentation is seen in untreated MCF-7 cells at 10 or 48 hr of the incubation. In a cell line that does not have the caspace-3 mutation, TNF-alpha induces chromatin fragmentation in mouse L929 fibroblasts upon 10 hr incubation. The data demonstrate the repair of the caspase-3 mutation and restoration of the apoptotic function in MCF-7 cells using human genomic DNA fragments.

7. Experiments were performed with a mouse model to determine if radiation sickness development induced by radiation exposure can be inhibited *in vivo* by the administration of genomic DNA fragments in accordance with the disclosure in the above-identified application. The mouse model is most widely used in radiation damage and protection research. Lethal levels of ionizing radiation cause massive amounts of mutations leading to the death of the cells and eventually the organism. Female CBA mice four months old were used in the experiments. The mice were irradiated with the intensity of 1.3 Gy/min with a total dose of 9.1 Gy, equal approximately to LD_{100} doses. Different treatments were applied to four groups of mice. The first, the control group, received only a placebo treatment of daily injections of physiologic saline starting 30 minutes post irradiation for 3 days. The second group received 1 mg of fragmented human genomic DNA on the first day and 0.5 mg on the second and third day starting 30 minutes after irradiation. The human placenta genomic DNA was fragmented by mildly sonication to yield fragments 200-3000 bp. The third group received 1 mg of fragmented CBA mouse genomic DNA 30 minutes before the irradiation. The CBA mouse genomic DNA was fragmented by mildly sonication to yield fragments 200-3000 bp. The fourth group received 1 mg of fragmented CBA mouse genomic DNA on the first day and 0.5 mg on the second and third days starting 30 minutes after irradiation. The CBA mouse genomic DNA was isolated from liver, kidney, and spleen of CBA male mice and fragmented by sonication. The data from the experiment is shown in Exhibit 4. All mice in the control group died in less than 20 days post-irradiation. The human DNA had some effect, although after 20 days less than 20% of the mice survived. In

contrast, over 40% of mice treated with mouse DNA before irradiation survived and over 60% of those treated with mouse DNA post-irradiation survived after 20 days. The surviving mice appeared healthy, although their hair became light grey in color. The data indicate that administration of genomic DNA in accordance with the above-identified application reduces the effects of ionizing irradiation on mice. The survival rate of the mice injected with a substantial amount (1 mg the first day, 0.5 mg second and third days) of DNA 30 minutes after lethal irradiation was significantly greater than that of the untreated mice.

8. Experiments were performed with a mouse model to determine if genotoxic effect due to exposure to chemical mutagens can be inhibited *in vivo* by the administration of genomic DNA fragments in accordance with the disclosure in the above-identified application.. In these experiments, mice that were exposed to the popular chemotherapeutic and mutagen cyclophosphamide develop strong leukopenia. The model is used to test potential modulators of the recovery. A/Sn mice were used in the experiments. The exposure of mice to cyclophosphamide results in a reduced number of circulating leukocytes. Groups of 12 male A/Sn strain mice were injected with cyclophosphamide (0.2 mg per 1g of weight). Fragmented human or murine genomic DNA with fragment lengths of 200-3000 bp was injected daily for 8 days at a dose of 50 μg per animal starting one day prior to the cyclophosphamide injection. Fragmented human genomic DNA was prepared from human placenta genomic DNA that was fragmented by mildly sonication to yield fragments 200-3000 bp. Fragmented CBA mouse genomic DNA was prepared from CBA mouse genomic DNA from liver, kidney, and spleen of CBA male mice. The CBA mouse genomic DNA was fragmented by mildly sonication to yield fragments 200-3000 bp. The control group received daily injections of saline. The data from the experiment are shown in Exhibit 5. The data demonstrate that mice injected with fragmented CBA mouse genomic DNA had lymphocytes return to normal levels after 7 days whereas the mice treated with saline or fragmented human genomic DNA returned to 50% of the normal level. The data indicate that administration of genomic DNA in accordance with the disclosure in the above-identified application reduces the effects of chemical mutagens on mice.

9. Clinical studies have been initiated to test the use of fragmented human genomic DNA administration in accordance with the disclosure in the above-identified application on human patients with cancer. The clinical studies are ongoing and have not been completed. Patients in these studies are selected with late stages (stage III or stage IV) of breast or ovarian carcinoma. The patients continue to undergo conventional medical treatment. Stages of disease are determined in accordance with standard medical practice. For example, stage IV is the last stage of ovarian cancer. It is characterized by cancer growth involving one or both ovaries with distant metastasis of pleural, liver and other locations. Patients in the studies are administered a Panagen preparation. Panagen which is fragmented human genomic DNA prepared from human placenta genomic DNA was prepared as follows. The human placenta genomic DNA was isolated from placentas and fragmented by mildly sonication to yield fragments 200-3000 bp. The placentas from which the human genomic DNA was isolated were selected exclusively from young women under 35 without chronic diseases and pathologies who delivered without pathology. Women donors were preliminary tested negative for HIV, syphilis, diabetes, hepatitis B and C. Preparations were tested for bacterial contamination and presence of DNA sequences of major pathogens in a certified medical laboratory. Panagen preparation was in the form of dried powder or tablets with enteric coating.

10. Stage IV Ovarian carcinoma is the last stage of ovarian cancer and is characterized by cancer growth involving one or both ovaries with distant metastasis to liver and pleural cavity. The standard medical treatment involves removal of the uterus, both fallopian tubes, both ovaries and omentum by surgery. After recovery from surgery, combination chemotherapy is provided, usually for 4 to 5 months. The 5-year survival rate for Stage III is 20 to 40%. The 5-year survival rate for Stage IV is about 11% (ACS: Treatment for Epithelial Ovarian Cancers by Stage). The 5-yr survival rate for Stages III and IV is 5 to 40% according to the Merck manual, Sec.18, Ch. 241, Gynecologic Neoplasms. Advanced-stage ovarian cancer usually recurs. Response to chemotherapy is assessed by measuring the marker CA 125. After completion of chemotherapy, a second-look laparotomy may be needed because about 2/3 of patients with stage III or IV disease have pathologically proven residual disease even after a complete clinical response to chemotherapy. Practically all

patients experience side effect symptoms during the chemotherapy treatments. Chronic fatigue, weakness, dizziness, irritability, shortness of breath, and chills are developed by over 76% of chemotherapy patients. Another common symptom is loss of immunity, caused by inhibition of leukocyte production, resulting in susceptibility to banal viral or bacterial infections that are normally not dangerous, but can be often fatal for chemotherapy patients. Chemotherapy normally causes ulceration of mouth and intestine mucosa, bleeding of gums and even a minor wound tends to be non-healable as a result of the therapy.

11. As mentioned above, the studies are ongoing and have not been completed. One patient with stage IV ovarian cancer enrolled in the clinical studies has completely recovered. In that case, the patient was a 63-year-old woman diagnosed with Stage IV ovarian cancer with metastases in the liver, omentum and regional lymphoid nodes. This patient underwent ovariectomy, extirpation of uterus on April 24, 2002 followed by 8 courses of chemotherapy with cyclophosphamide i/m 3 g per course and platine i/v. 110 mg per course over the period of time from May 1. 2002 to January 30, 2003. Panagen preparation was taken during the breaks between chemotherapy courses in the form of enemas have 5mg of the fragmented DNA mixed with 100 ml water containing 50 mg baking soda 6 times a day for 4 months. Then the regimen was changed to alternating 3 enemas + 3 tablets (5 mg) of Panagen 6 times a day for 4 months. Then the regimen was changed to just 6 tablets (5 mg) per day for 2 months. The Ovarian cancer marker CA 125 expression reduced from 300 U/ml on August 9, 2002 down to 3 U/ml on January 23, 2003. Echogenic cancer nodules 1cm in diameter located in liver 5^{th} segment were detected as metastasis and later disappeared as shown by ultrasound examination of the abdomen performed April 4, 2002 and August 28. 2002. An ascite 10 cm X 15 cm located in small pelvis was detected and later disappeared as shown by ultrasound examination of the abdomen and genito-urinary system performed April 1, 2002 and August 8, 2002. No commonly developing chronic fatigue associated with anemia occurred as a result of chemotherapy treatments. No usual loss of immunity associated with low leukocyte level was detected. No usual ulceration or vascular damage appeared. The patient returned to premorbid style of life after the treatment.

12. I declare that statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 04.24.2003

By: Leonid A. Yakubov
Dr. Leonid A. Yakubov

Exhibit 1: A copy of *curriculum vitae* of Dr. Leonid A. Yakubov

Exhibit 2: Data from experiments performed with a mouse tumor model

Exhibit 3: Data from experiments performed with caspase-3 mutated cultured cells

Exhibit 4: Data from experiments performed with a radiation sickness mouse model

Exhibit 5: Data from experiments performed with a chemical mutagen mouse model